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Identification of the presence of antibiotic residues in the water environment in Ndjamena, Chad

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Abstract

Objective: The objective of this study is to determine the presence of antibiotic residues in the water environment.

Methodology and results: It was a descriptive study with an analytical purpose from May 2022 to August 2022 carried out in 8 districts of the city of N'Djamena. Wastewater samples were collected and analyzed using standard food microbiology methods. The detection of antibiotic residues was done by the premiTest which is a qualitative detection tool. A total of 42 wastewater samples were taken in the various districts of the capital. The prevalence of antibiotic residues obtained was variable (50 to 100%) from a district to other. It was 100% in the 6th district, 83% in the 8th district, 58% in the 7th district and 50% in the 10th, 1st, 2nd, 3rd and 9th districts of the city of Ndjamena respectively.

Conclusion: The prevalence of antibiotic residues detected in the water environment reflects the poor management and use of antibiotics by human and veterinary health professionals in the city of N'Djamena. In this regard, the effluents generated by hospital and veterinary activities present a real danger for humans, animals and the environment, given the nature and importance of the specific substances they contain and because of their discharge. In the same way as conventional urban waste, into the communal sewerage system without prior treatment. The Ministry of Public Health and Prevention as well as the Ministry of Livestock and Animal Production must advocate to oblige officials at different levels to prohibit the discharge of drug residues into the environment of rational wastewater treatment in order to avoid bioresistance mechanisms in Chad.

Keywords: Identification**;** Antibiotic residues; Water environment; N'Djamena

1 Introduction

An antibiotic is an organic chemical substance of natural or synthetic origin that inhibits or kills pathogenic bacteria in low concentrations and has selective toxicity, as well as any product that can be administered to humans or animals for medical diagnosis or to restore, correct or modify their organic functions (Ronald and al., 2003).

The consumption of antibiotics in the world is estimated between 100,000 to 200,000 tons per year, and about 50% are used in veterinary medicine. Global consumption of antibiotics for the sole benefit of humans increased by 36% between

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2000 and 2010, similarly increasing the entry of antibiotic residues into the environment (Zabczynski and al., 2008; Duclair and al., 2014). Indeed, approximately 50-80% of the antibiotics consumed are directly excreted by our bodies and wastewater disposal systems do not completely degrade them (Kolpin and al., 2004; Kouadio and al., 2006). Antibiotic concentrations measured in freshwater, although mostly below clinically effective concentrations, are highly likely to have direct and indirect effects on the microbial component of aquatic communities. Indeed, even at low concentrations, these antibiotic residues could have significant consequences for ecosystems and for human and animal health. Antibiotics are specifically administered to treat infections or to increase yields particularly in livestock and agriculture, but in the environment other living organisms that are part of ecological processes, such as nutrient cycling, are inevitably exposed (Danner and al., 2019). The purpose of this work is to determine the presence of antibiotic residues in the water environment that had not yet been conducted in Chad.

2 Material and methods

2.1 Study areas

Our sampling was carried out in hospitals, retention basins, the Chari and Logone rivers and drainage channels. These samples were taken in eight (8) of the ten (10) districts of the city of N'Djamena, which include fifteen (15) quarters, including Farcha, Klemat, Diguel, Diguel Est, Zafaye, Amtoukoui, Chagoua, Dembé, Ardepdjoumal, Abena, Ngueli, Walia, Ndjari, Gardolet and Gosator.

2.2 Sampling

Sampling was non-probability and purposive. It began with a census of the sites and was followed by sampling in the hospitals, drainage canals, retention basins and the Abena slaughterhouse, as well as at the Farcha refrigerated slaughterhouse and on the Chari and Logone rivers.

2.3 Wastewater collection method and data

The wastewater collection technique: The data was collected using a questionnaire during an interview with the municipal officials in charge of water and the officials in charge of hygiene and sanitation in the health facilities. The samples were taken in a sterile flask with a capacity of 75 cL at depth (turned the flask upside down then introduce it to the bottom of the water and turned over, maintaining it until it was filled) and at the surface (tilted the flask then placed on the surface of the water and press lightly to fill) retention basins and channels then placed in an isothermal cooler and sent to the Microbiology laboratory service of the Institute of Livestock Research for Development (IRED) for analysis.

2.4 Analysis of the water samples: Search for antibiotic residues

2.4.1 Principle of PremiTest

PremiTest is based on the inhibition of the development of Bacillus stearothermophilus, a micro-organism very sensitive to many antibiotic residues and to sulfonamides. A standardized number of spores is embedded in an agar vehicle containing selected nutrients. When the test sample is added to a PremiTest ampoule and incubated at 64°C, the spores will germinate. These germinated spores will multiply and acidify the medium in the absence of the inhibiting substances. This results in a change of color of the indicator from purple to yellow. If the antimicrobial residues are present in sufficient quantity (above the MRL), the germ will not develop and the color will remain purple.

2.4.2 Procedure for processing water samples

From each water sample, 100µl was taken and placed in the test vials and left at room temperature for preincubation for 20 min. The water was then carefully removed from the ampoules by rinsing with deionized water. The ampoules were covered with a film supplied with the kit and incubated at 64°C for 2h40min. However, a negative control was carried out in parallel and made it possible to stop the incubation as soon as it stained complement.

2.4.3 Reading the results

According to the manufacturer, the reading of the "presence/absence" result is limited to a comparison of colors.

- In the absence of antibiotics, the spores germinate and grow, resulting in acidification of the medium and a color change from purple to yellow, this means that the amount of antimicrobial compounds is below the detection limits of the PremiTest.

- In the presence of antibiotics, the spores do not develop, they will be inhibited by the antibiotic and indicates a purple color indicates a level of antibiotics greater than or equal to the detection limit of the test.

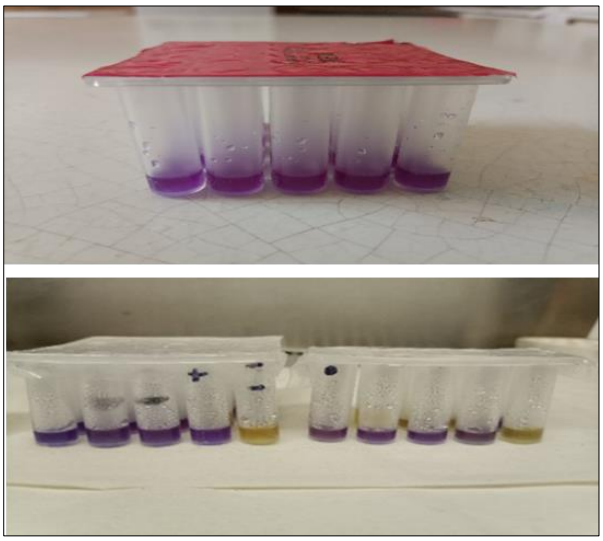

Figure 1 PremiTest used for the detection of antibiotic residues in the water environment: on the left is the premiTest kit; on the right is the result

2.4.4 Data processing and analysis

The data from the interviews as well as the results of the water samples tested were entered into a Microsoft Office 2016 Excel spreadsheet then converted to CSV then exported to R Studio software version 4.0.4.2021 for the analyzes. Regarding the analytical statistics, the Chi-square test and Fisher's exact test were used to compare the proportions (districts and quarters) and for their significance. The significance threshold was set at 0.05 and the p-value calculated using Fisher's Exact Test.

3 Results and discussion

Table 1 shows that of the 42 samples analyzed, 27 showed the presence of antibiotic residues, with prevalence of 64.3% (95% CI = [40.61; 87.99] and very significant at the threshold of 5% (p-value = 0.0003983).

Table 1 Prevalence of detection of ATB residues in the city of N'Djamena

Factors	ATB	Numbers	P rate in %	95% CI	P-value
Antibiotic detection	Presence	27	64.3	[40.61 ; 87.99]	0.0003983
	Absence	15	35.7	[-45.37 ;26.03]	
	Total	42	100		

Table 2 shows a variation in the prevalence of detection of antibiotic residues in the Arrondissement of the city of N'Djamena; the 6th and 8th Arrondissement recorded the highest rates with 100% and 83.33% respectively, but this variation is not significant at the 5% threshold (p-value = 0.1077).

Table 3 shows the variation by district, Amtoukoui, Ardepdjoumal, Diguel and Diguel Est each recorded the highest rate of 100% but this variation is not significant at the 5% threshold (p-value = 0.1077).

According to Table 4, the variation in the detection of antibiotic residues in the non-treatment of water shown by the study (85.7%) is very significant (95% CI = [49.14; 122.26] and very significant at the 5% level (p-value = 0.0003983).

Table 2 Prevalence of detection of ATB residues by arrondissement

Districts	Numbers	Presence/ Rate P in %	Absence/ Rate P in %	95% CI	P-value
10th	4	2 (50)	2 (50)	[-117.17; 217.17]	0,1077
1st	6	3 (50)	3 (50)	[-63.16; 163.16]	
2nd	4	2 (50)	2 (50)	[-117.17; 217.17]	
3rd	4	2 (50)	2 (50)	[-117.17; 217.17]	
6th	2	2 (100)	0 (0)	[-870.20;1070.20]	
7th	12	7 (58.33)	5 (41.67)	[-13.11; 129.77]	
8th	6	5 (83.33)	1 (16.67)	[-161.94; 217.17]	
9th	4	2 (50)	2 (50)	[-117.17; 328.60]	

Table 3 Prevalence of detection of ATB residues by neighborhood

Quarters	Numbers	Presence/ Rate Pin %	Absence/ Rate Pin %	95% CI	P-value
Abena	2	1 (50)	1 (50)	[-289.50; 389.50]	0,1077
Amtoukoui	2	2 (100)	0 (0)	[-870.20; 1070.20]	
Ardepdjoumal	2	2 (100)	0 (0)	[-870.20; 1070.20]	
Chagoua	6	4 (66,66)	2 (33.34)	[-108.36; 241.68]	
Clemat	4	2 (50)	2 (50)	[-117.17; 217.17]	
Dembé	2	2 (100)	0 (0)	[-870.20; 1070.20]	
Diguel	2	2 (100)	0 (0)	[-870.20; 1070.20]	
Diguel-Est	2	2 (100)	0 (0)	[-870.20; 1070.20]	
Farcha	6	4 (66.66)	2 (33.34)	[-108.36; 241.68]	
Gardolet	4	2 (50)	2 (50)	[-117.17; 217.17]	
Gozator	2	2 (50)	2 (50)	[-117.17; 217.17]	
Ndjari	2	2 (50)	2 (50)	[-117.17; 217.17]	
Nguéli	2	2 (50)	2 (50)	[-117.17; 217.17]	
Walia	2	2 (50)	2 (50)	[-117.17; 217.17]	
Zafaye	2	1 (50)	1 (50)	[-289.50; 389.50]	

Table 4 Variations related to water treatment and collection locations

Factors	Treatment	Numbers (N)	P rate in %	95% CI	P-value
Water Treatment	Yes	6	14.3	[13.15; 15.44]	0.003066
	No	36	85.7	[49.14; 122.26]	
	Total	42	100		

4 Discussion

In our study, considering our results related to the use of drugs, we can say that the positive samples contain antibiotic residues. But the method used does not allow us to identify the nature of the molecule present, nor to quantify it.This antibiotic residue study is justified by the fact that antibiotics are used in both human and veterinary medicine. Contamination of the external environment is possible and constitutes real environmental and health risks, in particular through the problem of bacterial resistance. The prevalence of antibiotic residues obtained in this study has a rate of 64.3%, it is significant at the 5% threshold (p-value = 0.0003983). This prevalence varies according to the districts and quarters.This study showed a non-significant variation at the 5% level in the districts. The 100% prevalence in the 6th arrondissement can be explained by the fact that the arrondissement is home to one of the largest canals in the city of N'Djamena (ardepdjoumal canal). Due to its depth and length, this canal is used by the agents of the N'Djamena City Hall and the residents as a dumping ground for biomedical waste from private practices and household waste, hence the confirmation of the presence of antibiotic residues. It should also be recognized that the effluents generated by hospital activities can present a potential danger to humans and their environment in this district. The prevalence is 83% in the 8th district, which can be translated by the fact that it groups together several districts with open defecation and the passage of animals, the presence of a large Chad-China Friendship hospital, whose purification station is not functioning. The presence of certain hotels in the area where all the liquid waste is drained into these channels can only be sources of propagation of antibiotic residues. The 7th arrondissement is a vast district with 5 wastewater retention basins, an animal slaughterhouse, three medical centers with fewer activities that drain their waste into the basins, and a prevalence of 58%. This rate is relatively lower than that of the 6th and 8th districts. The other districts 10th, 1st, 2nd, 3rd and 9th each recorded a prevalence of 50%. This rate is explained by the fact that in the 10th district there is only one retention basin which is fed by the district hospital of Gozator. In the 1st on the other hand, it also has a Peace Hospital in Farcha, a drainage canal fed by the waste from the base which was well treated and one of the largest functional slaughterhouses in Chad (Abattoir Frigorifique de Farcha). This large slaughterhouse rejects the waste directly into the river without any prior treatment. In the 2nd, we have the klemat basin which is fed by a private clinic SOS Village and the holy Martin Canal fed by household waste. The 3rd recorded only the big hospitals such as the University Hospital Center the National Reference which has a purification station works and the waste is well treated before being thrown and University Hospital Center the Mother and the Child also has a purification station but defective. In the 9th district, we only recorded the Logone River, which also empties its contents into the lake, so there is less chance of finding antibiotic residues.

For the variation according to neighborhoods, the prevalence was 100% in neighborhoods such as Abena, Amtoukoui, Ardepdjoumal, Dembé, Diguel Est and Diguel. This is due to the fact that these neighborhoods have the most polluting canals, retention basins and hospitals whose treatment systems are defective Several authors have reported similar cases, such as Brandt and al. (2015), which indicate that antibiotics play an important role in the management of infectious diseases in humans and animals worldwide. Antibiotics are produced, consumed, and released into the environment and this is of concern to the scientific community (Tamtam and al., 2009; Phillips and al., 2010). Antibiotic residues are found in hospital tributaries and reach sewage treatment plants and water environments (Spiroux, 2009) through several routes of antibiotic entry into freshwater: wastewater treatment plant effluents, chemical manufacturing plants, and livestock, agriculture, and aquaculture sites (Kim and al., 2011; Gottschall and al., 2012; Gibs and al., 2013). However, our result is still 43.3% higher than that reported by Souleymane and al. (2022) on antibiotic residues in meat and eggs sold in Chad. This can be explained by the fact that wastewater is not treated before disposal, including health facilities (85.7% do not treat wastewater). On the other hand, our result is close to the one found (51%) by Kantati (2011) on the population of cattle slaughtered in Dakar slaughterhouses with the same method used, namely the PremiTest. This prevalence of 64.3% is lower than that obtained by Ibrahim (2016) of 70.55% in Cameroon. In the case of our study, a number of reasons can explain the high prevalence of antibiotic residues in the water environment established by Gorman and al. (2013) stipulates that inappropriate management of these wastes at the level of hospital facilities increases the infectious, toxic, radioactive and psycho-emotional risks for health professionals and hospital users because health facilities in Chad do not have appropriate purification stations. A study of WHO (2002) on 22 developing countries showed that 18-64% of health facilities do not dispose of their waste properly. This presence of antibiotic residues in wastewater from a treatment plant can alter bacterial communities and cause resistance to develop in surrounding bacteria.Hospitals discharge a high volume of wastewater, with variable physicochemical compositions, including chemical and biological products, pharmaceutical toxic substances, radioactive elements and pathogenic microorganisms into urban drainage channels, into retention basins, into rivers and then into the lake including consumer waste. For veterinarians, this concerns both pets and livestock for curative, preventive or zootechnical purposes. The elimination of veterinary drugs by fecal or urinary route leads to their introduction into the environment, immediately when the animals are grazing, or later in case of spreading of manure. A particularity of veterinary drugs is the existence of treatments directly administered in the environment. This is the case of veterinary drugs intended for aquaculture, directly poured into the breeding ponds. This is also the case for dilution solutions for

sheep bathing: after use, the bath water is discharged into the surrounding environment (Aminata and al., 2018). The studies realized by Alabi and al. (2011) have shown that hospitals represent an undeniable source of release of many chemical compounds from the environment due to medical activity.

5 Conclusion

Our study determined the presence of antibiotic residues in the water environment, namely in health facilities, in urban drainage canals, in the retention basins of the city of N'Djamena with a prevalence of 64.3%. It is very important that the authorities of the municipality of N'Djamena, the actors in charge of public health and livestock should pay particular attention to the misuse of hospital waste, household waste, the use and anarchic sale of antibiotics or drugs and non-compliance with waiting times. The effluents generated by hospital and veterinary activity present a potential danger for humans and their environment given the nature and importance of the specific substances they contain and because of their disposal, in the same way as classic urban discharges, towards the municipal sewage network without prior treatment. Nevertheless, they should lead us to assess the health risk, improve the techniques and develop the methods necessary to limit the release of drug residues into the water environment.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest related to this study.

Statement of author's contributions

Design of the draft article produced by DM; Conduct of research activities by BBA; data collection carried out by DM, NKA, HHA; Processing of collected data carried out by DM, NKA, HHA, RBV; Statistical analysis of the data carried out by DM, RLG, NKA; writing of the article by DM; supervision of laboratory analyzes carried out by NBNR; participated in the writing of the article; reading and correction of the article produced by DM, BBA, NB, NKA, RLG, NS.

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